HIV-1/2 3.0 (Standard Diagnostics, Inc.), Uni-Gold™ Recombigen® HIV (Trinity Biotech plc), ARCHITECT™ (Abbott Laboratories), Determine® (Alere), and COBAS CORE Anti-HIV-1/HIV-2 EIA DAGS (CORE HIV-1/2) (Roche). See also Zaaijer et al., The Lancet, vol. 340, pp. 770-772, 1992, and Miolini et al., Journal of Immunological Methods, vol. 20, pp. 25-34, 1978). This technique has become known as the "third generation" of HIV antibody detection because it is capable of identifying a broader range of antibody types than previous "first" and "second" generation tests. The technique is also often incorporated into the current "fourth" generation HIV antigen/antibody combination assays.

[0184] The simple labeled antigen assay technique, as described herein, provides a number of advantages over existing technology. For example, current third- and fourth-generation HIV diagnostic assays that use the double antigen sandwich approach require integration into an immunochromatographic (e.g., lateral flow) device, which requires colorimetric visual interpretation, or into an expensive, fully-integrated, automated system with automated fluidic handling and optical readout. The labeled antigen assay, in the present embodiment, generates signal through fluorescence imaging of captured complexes with evanescent illumination. As described herein, the combination of labeled antigens, evanescent illumination, and fluorescent detection provides advantages of simplicity (i.e., minimal user interactions), speed (i.e., time to result), multiplexing (i.e., multiple assay results for a single sample) and compatibility with complex sample matrices (e.g., whole blood). In addition, the system described herein provides a means to perform assays based on kinetic data, enables numerous in-assay controls, and can provide means for automatically timing the assay. The approach also allows fluorescence signal acquisition of bound complexes on the surface without the need for washing away of residual unbound fluorescent markers in the liquid volume above the surface, thus allowing a truly one step process from biological sample introduction through signal acquisition. While a wash step may still be useful in some applications of the labeled antigen assay described herein, the potential elimination of the wash step may provide a significant advantage over existing assay protocols.

[0185] FIG. **39** shows a diagrammatic representation of a labeled antigen assay technique, in accordance with an embodiment, which is a further simplification of the aforementioned double antigen sandwich technique. As shown in FIG. **39**, an antigen is immobilized onto an assay surface. A labeled antigen, including the same antigenic epitope as the immobilized antigen, serves as the detect reagent. When the specific target antibody is present in the biological sample, a double antigen sandwich is formed at the assay surface.

[0186] FIG. **40** shows a flow chart, summarizing an exemplary labeled antigen assay process flow, in accordance with an embodiment. An assay process **4000** begins with an antigen immobilization step **4005**, in which one or more appropriate antigens as well as potentially positive and negative controls are immobilized on an assay surface, such as assay surface **2620** of FIG. **26**. Step **4005** may be performed, for example, by the manufacturer of the assay system rather than the assay system user.

[0187] Continuing to refer to FIG. **40**, assay process **4000** then proceeds to a step **4010**, in which a biological sample, such as a serum sample, is reacted with a labeled antigen mix. The labeled antigen mix may be provided by the assay system manufacturer or custom-formulated by the assay system user. The mixed sample is then added to a fluidic sample chamber in a step **4015**. Optionally, excess labeled antigen mix may be washed away from assay surface **2620** in an optional step **4018**. The fluorescence signal at the assay surface is then imaged by the assay system in a step **4020**, and then the captured image may be analyzed in a step **4025**.

[0188] The assay described with respect to this example may be reduced to a single step assay, in which the only user interaction is the introduction of biological sample to the assay device. In an embodiment, the labeled antigen mix may be immobilized within the fluidic sample chamber using conventional methods such as lyophilization. For example, the labeled antigen mix may be lyophilized along with sugar-based stabilizers at or near an inlet port of the assay system. Upon biological sample introduction, the labeled antigen mix is rehydrated and target antibody-labeled antigen complexes are formed. The complexes may then bind to the appropriate immobilized antigen sites on the assay surface, thereby forming the antigen-antibody-antigen complexes as previously described. A further advantage of this embodiment is that the sensitivity of assay system **2600** may allow elimination of subsequent wash steps. In particular, when using planar waveguide illumination, the evanescent field is localized within a few hundred nanometers of the assay surface for visible light illumination. Consequently, fluorescent dye in the bulk solution of fluidic sample chamber does not contribute to the fluorescence signal measured at detection system **2660**. The result is a true single step assay: a biological sample is added to cartridge **2602**, which is then imaged on detection system **2660** in step **4080** and subsequently analyzed in step **4025**. Alternatively, a final wash step **4018** may potentially yield improved signal-to-background performance in the assay and may therefore be useful in certain assay applications. Several methods for the final wash step may be envisioned. For example, this step may be a simple wash buffer addition introduced by the user from a dropper bottle. Alternatively, the final wash buffer may be stored on-board the device, such as in a blister pack that is either deployed by the user or automatically by activation in the detection system.

[0189] We note that the workflow outlined in FIG. **40** is only exemplary. Other embodiments may have different sequences of steps or additional modifications.

Example 10

Labeled Antigen Assay with Serum Samples

[0190] Array Printing: Recombinant antigens associated with human immunodeficiency virus ("HIV") and *Treponema pallidum* (causative organism of syphilis) were printed in duplicate as a geometrically defined array on assay surface **2620** of planar waveguide **2605** using a conventional arraying robot (Bio-Dot). gp 41 and HIV-1 p24 protein were printed for HIV infection analysis, while *Treponema pallidum* proteins p17 and p47 were printed for detection of syphilis antibodies.

[0191] Antigen labeling and labeled antigen mix formulation: Aliquots of the antigens printed to the array were covalently labeled with fluorescent dye Alexa-647 and quantitated by UV absorbance. Optimal working concentrations of labeled antigens were determined empirically, and a labeled antigen mix of antigens at two times the working concentration in assay buffer (1×=1× phosphate buffered